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PRELIMINARY STUDIES ON SEED GERMINATION OF *DROSERA ALICIAE* HAMET

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Abstract

Seed germination of the carnivorous plant *Drosera aliciae* occurred in a wide range of pH values with an optimum at pH 5,5. Germination proceeded at almost equally high rates in continuous light at 10°C, in the dark at 15°C and in a 14 hour photoperiod at 10/10°C and 15/10°C day/night temperature. Germination was stimulated slightly by KNO_3, H_3BO_3, gibberellic acid, indole-3-acetic acid and kinetin, the effect being greater with one year old seed than with fresh seed. An after-ripening period in the imbibed state of approximately 24 days was observed. None of the treatments, including dry storage of seed, reduced this period. This period could not be ascribed to an underdeveloped embryo. It is suggested that the after-ripening period is required for physiological changes to occur.

Uittreksel

VOORLOPIGE STUDIES OP DIE ONTKIEMING VAN *DROSERA ALICIAE* HAMET

Sade van die inseketende plant *Drosera aliciae* het oor 'n wye pH-gebied ontkiem met 'n optimum by pH 5,5. Ontkiemingstempo's was feitlik ewe hoog in konstante lig by 10°C, in die donker by 15°C en in 'n 14-uur fotoperiode by 10/10°C en 15/10°C dag/nag temperatuur. Ontkieming is effens deur KNO_3, H_3BO_3, gibberelliensuur, indool-3-asynsuur en kinetien gestimuleer. Die effek was groter met een jaar oue saad as met vars saad. 'n Na-rypingsperiode in die geïmbibeerde toestand van ongeveer 24 dae is waargeneem. Geen behandeling, insluitende opberging van saad in die droë toestand, het hierdie periode verkort nie. Hierdie periode kon nie aan 'n onderontwikkelde embrio toegeskryf word nie. Daar word voorgestel dat dit benodig word vir fisiologiese veranderings om plaas te vind.

Introduction

The carnivorous plant *Drosera aliciae* is, according to Obermeyer (1970), a marsh plant, also found in damp peaty areas and is common in the south-western Cape, extending to the eastern Cape. We have found it to occur abundantly in moist places in the Storms River—Knysna areas. First attempts at germinating seeds of this species on moist filter paper at 25°C over a four week period were unsuccessful.

As far as we could ascertain seed germination of this species has not been investigated. In fact carnivorous plants in general have been neglected in this respect. Swamy and Ram (1967) could not obtain germination of *Drosophyllum lusitanicum* seeds even after 8–10 weeks in culture. Excised embryos, however, germinated on a modified White's medium. Harder (1964) reported prompt

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germination in the same species but the condition of the seed and percentage germination was not given. Only the ovules from immature fruits of *Darlingtonia californica*, *Dionaea muscipula*, *Sarracenia flava*, and *S. purpurea* could be germinated by Withner (1964). Smith (1931) also states that the seeds of *Dionaea* are difficult to germinate. Vickery (1933) found seeds of *Drosera peltata* and *D. auriculata* to germinate readily in the laboratory on moist filter paper; seedlings commenced to appear after about 14 days.

This paper reports investigations on the effect of pH, temperature, light and various chemicals, including growth substances, on germination.

MATERIAL AND METHODS

Seeds were collected on various occasions from the same population of plants growing approximately three miles west of the Storms River bridge along the highway between Port Elizabeth and Cape Town. These were stored in open polystyrene containers in the laboratory.

Similar techniques were employed for all studies and for this reason only

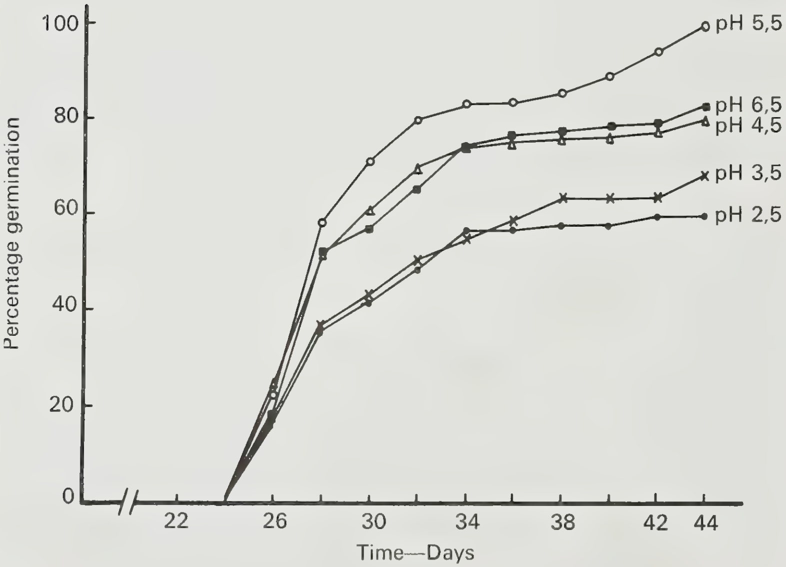

FIG. 1.
Time-course of germination at different pH-values. Mean of 5 replicates.

a general description is given here. Details on each experiment are presented in the section on results.

Prior to each experiment seeds were surface sterilized by submerging them in 95% ethyl alcohol for two minutes followed by three minutes in 0,1% mercuric chloride solution and rinsed thoroughly with a large volume of sterilized distilled water. All germination tests were performed on three layers of Whatman No. 1 filter paper in 9 cm oven sterilized glass petri dishes. In all cases 20 seeds were used per dish. Each treatment was replicated five times.

RESULTS

The effect of pH on germination of 5 *month old seeds*

Distilled water was adjusted to pH values of 2,0; 2,5; 3,0; 3,5; 4,0; 4,5; 5,0; 5,5; 6,0; 6,5; and 7,0 by small additions of 1N HCl or 1N NaOH. The filter paper on which seeds germinated was moistened with 5 ml of the above solutions. Seeds were exposed to weak light (2 000 lux) obtained from a combination of fluorescent tubes and incandescent lamps during 14 hours per day at 18–22°C in a growth room. The night temperature fluctuated between 9 and 12°C.

No germination occurred at pH 2,0. Although a fairly high germination count after 44 days was recorded at all other pH treatments, the highest germination rate occurred at pH 5,5 (Fig. 1). It is interesting to note that the first germinated seeds were observed only after 24 days.

The effect of temperature and light on germination of 6 *month old seeds*

Seeds were germinated in continuous weak light (2 000 lux), in the dark or in a light/dark cycle of 14 hours light and 10 hours dark per day. The continuous light and dark treatments were carried out at temperatures of 10°, 15°, 20° and 25°C in growth rooms. Fluctuating temperatures of 10/10°, 15/10°, 20/10° and 25/10°C were used in conjunction with the light/dark treatment. Deviation from the stated temperatures was of the order of ±0,5°C. Counts of germinated seeds in the "dark" treatments were made in "safe" green light. The filter paper on which seeds germinated was moistened with 5 ml of a solution of distilled water adjusted to pH 5,5.

The effect of the treatments on germination obtained on the 34th day of the experiment is shown in Fig. 2.

A marked interaction between temperature and light treatment was observed. Regardless of the light treatment, the higher temperatures retarded germination. In continuous dark, germination was also retarded at 10°C. This effect was overcome by exposure to light. On the other hand the retarding effect of 15°C in the light was overcome in the dark. It is interesting to note that the germination behaviour in the light/dark treatment at 10°C was similar to that in continuous light whereas at 15/10°C it was similar to that in continuous dark at 15°C.

At the lower temperatures an initial lag period of at least 23 days to onset of germination was also noted in this experiment. Where seeds were exposed to 25°C the first germinated seeds were recorded on the 33rd day.

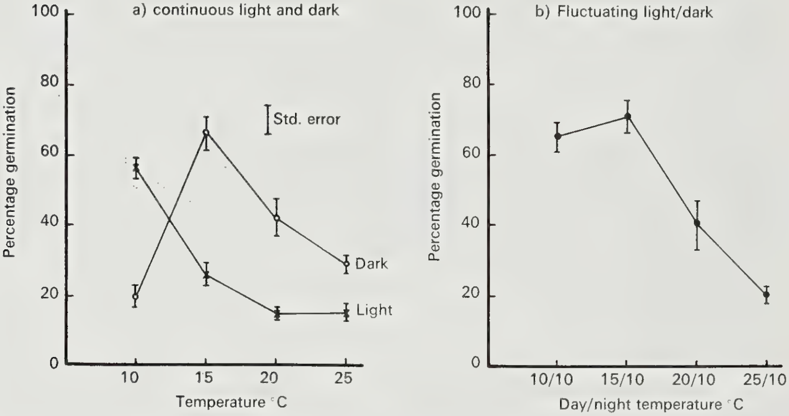

Fig. 2.
Effect of light and temperature on seed germination obtained on 34th day of experiment. Mean of 5 replicates.

The effect of certain chemicals on germination of fresh and one year old seed

Freshly harvested and one year old seed was germinated on filter paper moistened with 5 ml of solutions of KNO_3, H_3BO_3, indole-3-acetic acid (IAA—Nutritional Biochemicals corp), gibberellic acid (GA_3; Koch-Light) and kinetin (Koch-Light) at concentrations of 0,005; 0,05; 0,5 and 5,0 ppm. in 0,005 M phosphate buffer. The solutions had a pH of 5,5 after autoclaving. As controls, seeds were germinated in 0,005 M sterilized phosphate buffer, pH 5,5. The experiment was conducted in a Controlled Environments CE 7H growth cabinet set at a 14 hour photoperiod, 1 500 lux light intensity and a day/night fluctuating temperature of 15°/10°C. Germinated seeds were counted every second day for a period of 44 days.

As in the previous experiments, an initial lag period to onset of germination of approximately 24 days was found. No treatment shortened this period. The lag period was similar for both old and fresh seeds although the subsequent germination rate of old seed was markedly lower than that of fresh seed. No treatment was inhibitory to seed germination. In fact in most cases a slight stimulation was observed. For simplicity the germination obtained on the 34th day of the experiment of that concentration for each chemical which produced the highest stimulation is shown in Fig. 3.

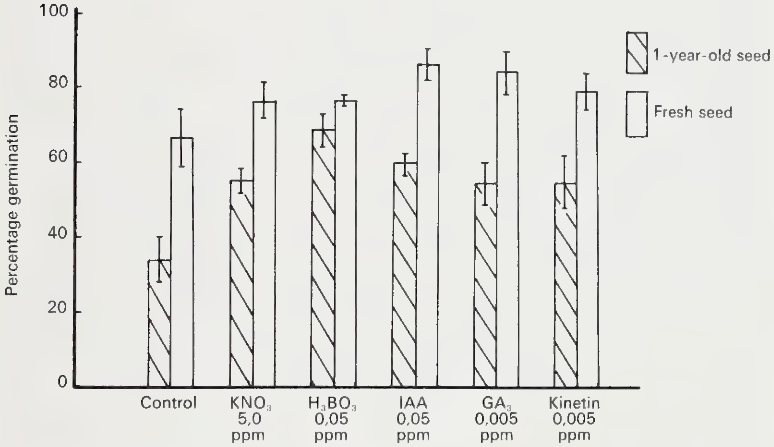

FIG. 3.
The effect of various chemicals on germination of 1-year old and fresh seed obtained on 34th day of experiment. Mean of 5 replicates.

Although all chemicals, at the concentration shown, stimulated germination, the effect on fresh seed germination was slight. The most marked effect was observed with old seed. Boric acid appeared to stimulate old seed germination more than any other chemical.

In addition to the treatments described above various other treatments were tested. These were: (i) soaking in tap water for 1 to 3 days, (ii) leaching in running tap water for 1 to 3 days, (iii) stratification at 5°C for 6–8 weeks, (iv) scarification in 50% H_2SO_4 for 1 to 15 minutes, (v) treating seeds with 98% ethanol for 1–15 minutes, (vi) germinating on filter paper moistened with 0,25 and 0,5% glucose and in other cases with the same sucrose concentration, (vii) exposing seeds on moist filter paper to an atmosphere of 100% oxygen for 3–5 days and (viii) soaking in a H_2O_2 solution (5–30%) for 24, 48 and 72 hours. With the exception of H_2O_2 none of these treatments had any effect on seed germination. In the case of H_2O_2 inconsistent results were obtained. In one out of three experiments soaking in 5% H_2O_2 stimulated germination slightly. In the other two experiments a highly inhibitory action was observed.

Observations on Embryo Structure.

In none of the experiments performed could seeds be induced to germinate promptly on imbibition. Invariably a period of approximately 24 days to onset of germination appeared to be required. To ascertain whether this was possibly due to an immature embryo, seeds which were set out for germination at 15/10°C in a 14 hour photoperiod were taken at one-day intervals, fixed in FAA, dehydrated with tertiary butyl alcohol (Johansen, 1940) embedded in wax and sectioned longitudinally. Sections were stained with safranin and fast green.

From an examination of a large number of ripe unimbibed seeds it appeared that the embryo was small but fully organized (Fig. 4a). On imbibition no morphological or size changes of the embryo were observed up to about the 17th day. At this stage the embryos of some seeds increased in size mainly due to anticlinal cell division in the region of the hypocotyl (Fig. 4b). Germination is finally attained by increased cell division in this region followed by cell division in the cotyledons (Fig. 4c and 4d).

DISCUSSION

From the results presented it is clear that seeds of *Drosera aliciae* do not germinate readily. None of the conditions tested induced visible signs of germination in less than about 24 days after the start of imbibition. This period was similar for fresh and old seed indicating that after-ripening does not occur during storage of seeds in the dry state. Subsequent germination rates of old seed was markedly lower than that of fresh seed.

A more severe condition has been reported for *Darlingtonia californica*, *Dionaea muscipula*, *Sarracenia flava* and *S. purpurea* by Withner (1964) in which mature seeds failed to germinate but ovules of immature fruits germinated readily. Similarly Swamy and Ram (1967) obtained germination only with excised embryos of *Drosophyllum lusitanicum*. Mature seeds failed to germinate even after 8–10 weeks. In contrast Vickery (1933) found seeds of *Drosera peltata* and *D. auriculata* to germinate readily on moist filter paper although it was stated that seedlings commenced to appear after 14 days.

From the results of the present investigation it appears that *D. aliciae* seeds require a period of after-ripening in the imbibed state; this period being

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FIG. 4.

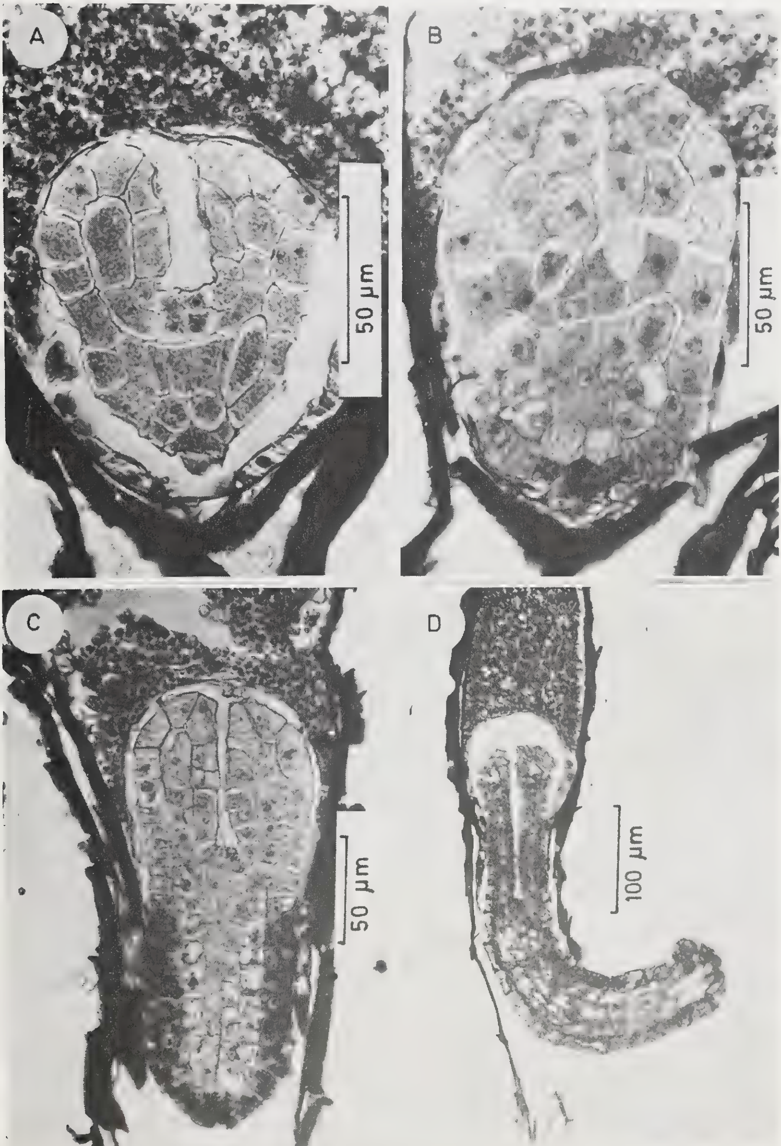
Longitudinal sections of germinating embryos of *Drosera aliciae*.

A—Embryo of ripe seed prior to imbibition.

B—Embryo showing anticlinal cell division in hypocotyledonary region after 17 days of imbibition.

C—Embryo imbibed for 21 days showing cell division in the cotyledons and hypocotyledonary region.

D—Protruding embryo 26 days after sowing.


A
50 µm
B
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C
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D
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shorter at a moderate temperature (15°C) than at a high temperature (25°C). Germinating seeds at 10°C or a pretreatment at 5°C for 6 weeks did not shorten this period. Since embryos appeared fully organized an after-ripening period for physiological changes to occur is suggested. A lack of water uptake did not appear to be responsible as sulphuric acid scarification had no effect on germination. Although impermeability of the seed coat to oxygen cannot be ruled out, this seems unlikely since incubating seeds in 100% oxygen for 3 to 5 days had no effect on germination behaviour.

An obvious interaction between temperature and light was observed. An interesting feature of these results was the finding that seeds exposed to a 14 hour photoperiod had equally high germination rates at 10°C and 15/10°C, whereas seeds kept in continuous light had highest germination rates at 10°C and those kept in the dark had highest rates at 15°C. Higher temperatures even if given only during the photoperiod were detrimental.

Although none of the chemical treatments induced prompt germination, increased germination rates subsequent to the initial after-ripening period were effected by KNO_3, H_3BO_3, IAA, GA_3 and kinetin. This effect was particularly pronounced in old seed. Furthermore H_3BO_3 had the greatest stimulating effect. This is interesting in view of the fact that Cresswell and Nelson (1972) have recently shown that boron is capable of breaking dormancy in *Themeda triandra* caryopsis and also stimulates germination of nondormant caryopsis. Only the "stimulating effect" was observed in the present study.

The stimulating effect of GA_3 on seed germination is well documented (Mayer and Poljakoff-Mayber, 1963). Although the majority of experiments testing the effects of IAA on seed germination have produced negative results, stimulation in a few cases has been observed (Lang, 1965). Kinetin effects on germination have mainly been associated with light-sensitive seeds (Evenari, 1965).

As a matter of interest it may be noted that distilled water slurries of various samples of soil in which plants were growing in the wild, had pH values of 5,3–5,8 which may have some bearing on the finding that the optimum pH for germination was 5,5.

ACKNOWLEDGMENTS

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REFERENCES

CRESSWELL, C. F. and NELSON, H., 1972. The effect of boron on the breaking, and possible control of dormancy of seed of *Themeda triandra* Forsk. *Ann. Bot.* **36:** 771–780.

EVENARI, M., 1965. Light and seed dormancy. In: W. Ruhland, (ed.) *Encyclopedia of Plant Physiology*. **15** (2): 804–847. New York: Springer-Verlag.

HARDER, R., 1964. Vegetative Entwicklung und Blütenbildung in axenischen Kulturen der insektivore *Drosera pygmaea* DC. *Planta* **63:** 316–325.

JOHANSEN, D. A., 1940. *Plant Microtechnique*. New York: McGraw-Hill.

LANG, A., 1965. Effects of some internal and external conditions on seed germination. In: W. Ruhland, (ed.) *Encyclopedia of Plant Physiology*. **15** (2): 848–893. New York: Springer-Verlag.

MAYER, A. M. and POLJAKOFF-MAYBER, A., 1963. *The Germination of seeds*. Oxford: Pergamon Press.

OBERMEYER, A. A., 1970. Droseraceae. In: L. E. Codd, *et al.*, (ed.) *Flora of Southern Africa*, vol. 13, Pretoria, Government Printer.

SMITH, C. M., 1931. Development of *Dionaea muscipula* 2. Germination of seed and development of seedling to maturity. *Bot. Gaz.* **91:** 377–394.

SWAMY, R. D. and RAM, H. Y. M., 1967. Cultivation of embryos of *Drosophyllum lusitanicum* Link—an insectivorous plant. *Experientia* **23:** 675.

VICKERY, J. W., 1933. Vegetative reproduction in *Drosera peltata* and *D. auriculata*. *Proc. Linn. Soc. N.S.W.* **58:** 245–269.

WITHNER, C. L., 1964. Notes on the cultivation of insectivorous plants in vitro. *Bull. Torrey bot. Club*. **91:** 412–413.